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CHAPTER 9

Cytokines in Autoimmunity

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Autoimmunity is regarded as the inability of the immune system to distinguish between self and nonself. The immune system has the capacity to maintain a state of equilibrium, although it responds to a diverse array of foreign antigens and despite its permanent exposure to self antigens. During the past decade, much progress has been made in the understanding of processes that lead from a normal autoimmune state, in which no clinical manifestations exist, to an autoimmune disease. It has been hypothesized that, after an infection, immune response spreads to tissue-specific autoantigens in genetically predisposed subjects, eventually determining progression to disease. Molecular mimicry between microbial or viral and self antigens may in some instances initiate autoimmunity. Local release of inflammatory cytokines after infection probably plays a pivotal role in determining loss of tolerance to self autoantigens and the pathogenic activation of autoreactive cells (1–3).

T-cell tolerance to self antigens cannot solely be accounted for intrathymic clonal deletion or induction of anergy in peripheral T cells. In several experimental systems, expression of the pathogenic capacity of potentially self-reactive T cells was actively prevented by other T cells with an immunoregulatory role (4,5).

Primary exposure of naive $CD4^+$ T cell to antigen results in differentiation to a defined helper subset. The preferential development of a particular helper T-cell subset correlates directly with susceptibility or resistance to certain disease states. Various factors influence the differentiation of particular T_H response, including antigen type, antigen dose, the type of antigen-presenting cell (APC), and the presence of cytokines. The primary helper T-cell types are T_H1 and T_H2, which are characterized by distinct patterns of cytokine secretion.

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This chapter considers cytokine participation in the pathogenesis of autoimmune diseases by analyzing evidence for their participation in these diseases. Each disease shows some similar and some distinct features, some of which are shared with other autoimmune diseases (5–8). Figure 9.1 shows some of the immunoregulatory networks that may be pertinent to the understanding of autoimmune disease.

T_H1 AND T_H2 T-CELL SUBSETS

Among the factors known to play an important role in determining the T_H1-T_H2 balance are antigen and costimulation. Low and high antigen concentrations tend to induce T_H2 responses preferentially, whereas intermediate doses induce T_H1 responses. The mechanism responsible for these effects is poorly understood. It is possible that certain concentrations of antigen induce a state of tolerance that preferentially shuts off T_H1 responses (9–11).

During the presentation of antigens, the type of APC also has a profound effect on the resultant immune response. Langerhans cells and dendritic cells induce T_H1 proliferation, whereas B cells tend to induce a T_H2 response (12). A major factor in this dichotomy in APC function is the elaboration of particular cytokines on antigen encounter (13).

T_H1 cells secrete interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) and are involved in cell-mediated inflammatory responses. T_H2 cells secrete IL-4, IL-5, IL-6, IL-8, IL-10, and IL-13 and favor humoral responses and allergy (14). An important feature of these helper T-cell subsets is the ability of one subset to regulate the activities of the other. The balance between these cells can play a major role in the type of disease manifestation observed. The establishment of this balance depends on many factors, including the antigen structure, the APCs, and the cytokine environment. This feature is critical for understanding the adverse effects induced by cytokines. There is much evidence that the cytokine products of each helper T-cell subset inhibit the differentiation and effector functions of the other. For example, IFN-γ has been shown to prevent T_H2

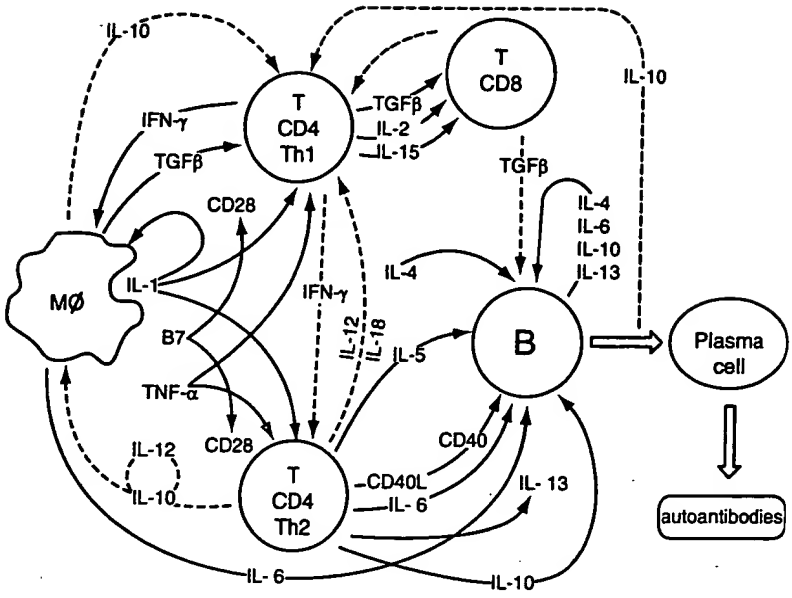

FIG. 9.1. Immunoregulatory pathways in humans. Interplays of various activation *(solid lines)* and inhibition systems *(dashed lines)* are shown. Many cytokines have stimulating or inhibitory activities that are influenced by the microenvironment of a cell. Several cytokines are inhibited by soluble receptors; some cytokines inhibit other cytokines.

cell proliferation, whereas IL-10 inhibits the synthesis of T_H1 cytokines (14,15). Emergence of a T_H2-type response typically results in the inhibition of T_H1 differentiation and the downregulation of T_H1-mediated immune responses.

CURRENT CONCEPTS OF CYTOKINES

Cytokine is a generic name for a diverse group of soluble proteins and peptides, which act as regulators at low concentrations (nanomolar to picomolar) and which, under normal or pathologic conditions, modulate the functional activities of individual cells and tissues. These molecules also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. The biologic activities of cytokines can be measured by a variety of bioassays employing factor-dependent cell lines or antibodies (enzyme-linked immunoabsorbent assay [ELISA]); using modern techniques of molecular biology, it is possible to measure message amplification phenotyping and to detect the presence of cytokine-specific mRNAs (16).

Many genes encoding cytokines can give rise to a variety of forms of cytokines by means of alternative splicing, yielding molecules with slightly different but biologically significant bioactivities. The expression patterns of different forms of cytokines or of members of a cytokine family are overlapping only partially, suggesting a specific role for each factor.

Almost all cytokines are pleiotropic effectors showing multiple biologic activities. Several cytokines have overlapping activities, and a single cell frequently interacts with multiple cytokines with similar responses. A consequence of this functional overlap is that one factor frequently may functionally replace another altogether or at least partially compensate for the lack of another mediator. Because most cytokines have ubiquitous biologic activities, their physiologic significance as normal regulators or in pathologic situations is often difficult to assess (17,18). Table 9.1 lists the cytokines and their main functions.

Cytokines show stimulating or inhibitory activities and may synergize or antagonize the actions of other factors. A single cytokine may also under certain circumstances elicit reactions that are the reverse of those under other circumstances. The type, the duration, and the extent of cellular activities induced by a particular cytokine can be influenced considerably by the microenvironment of a cell, depending, for example, on the cell cycle phase, the type of APCs, cytokine concentrations, the combination of other cytokines present at the same time, and even on the temporal sequence of several cytokines acting on the same cell. Cytokine signals can travel quickly to remote areas of a multicellular organism. They can reach numerous target cells and can be degraded quickly. It can be assumed that cytokines play an important role in all sorts of cell to cell communication processes, although many of the mechanisms of their actions are still unknown. They also play a central role in neuroimmunologic, neuroendocrinologic, and neuroregulatory processes. Cytokines are important regulators of mitosis, differentiation, migration, cell survival, and cell death. Several infectious agents exploit the cytokine repertoire of organisms to evade immune responses of the host (19,20).

Viruses appear to affect the activities of cytokines by inhibiting the synthesis and release of cytokines from infected cells, by interfering with the interaction between cytokines and their receptors, by inhibiting signal transmission pathways of cytokines, and by synthesizing virus-encoded cytokines that antagonize the effects of host cytokines mediating antiviral processes. The biologic activities of cytokines are mediated by specific membrane receptors, which can be expressed on virtually all cell types. Some receptors are expressed constitutively, but their expression is also subject to several regulatory mechanisms (20).

TABLE 9.1. *Cytokines and their main functions*

Cytokine	Producer cells	Main actions
IL-1 α/β	Monocytes/macrophages	Induces prostaglandin synthesis by endothelial cells
	Endothelial cells	Induces collagenase synthesis in sinovim and cartilage
	Fibroblasts	T-cell activation, IL-2 production, and RIL-2 expression
	Neurons	Induces GM-CSF and IL-4 production by activated T cells
	Glial cells	Stimulates B-cell proliferation and differentiation
	Keratinocytes	Synergistic action with other cytokines activating NK cells
	Epithelial cells	
IL-2	T cells	T-cell proliferation and differentiation
		Stimulates cytolytic activity of NK cells
		Stimulates B-cell proliferation and Ig synthesis
		Associated with its receptor, it is involved in signals transduction
		Activates NK and LAK cells
		Induces cell-mediated immune responses
		Stimulates neutrophil and macrophage functions
IL-3	T cells	Stimulates growth and differentiation of myelomonocytic lineage
	Thymic epithelial cells	Stimulates erythroid progenitors
	Keratinocytes	
	Neurons	
	Mast cells	
IL-4	T cells	Induces naive T-cell differentiation to T_H2 cells
	Macrophages	B-cell growth and differentiation
	Mast cells	Promotes isotype switch (IgG4 and IgE)
	Basofils	Stimulates endothelial cells and fibroblasts
	B cells	
	Bone marrow stroma	
IL-5	T cells	Eosinophil growth and differentiation
	Mast cells	Chemotactic for eosinophils
IL-6	T cells	Activates hematopoietic progenitor cells
	Monocytes/macrophages	Induces megakaryocyte development
	Fibroblasts	Induces growth and differentiation of T and B cells, hepatocytes and keratinocytes
	Hepatocytes	Stimulates acute-phase protein production by hepatocytes
	Endothelial cells	
	Neurons	
IL-7	Bone marrow stroma	Growth of pre-B and pro-B cells
	Fetal liver cells	T-cell proliferation
		NK and LAK cell enhancing activity
IL-8	Monocytes	Chemotactic for neutrophils, T cells, and basophils
	T cells	Induces release of lisosomal enzymes
	Fibroblasts	Induces neutrophil adhesion to endothelial cells
	Endothelial cells	
	Keratinocytes	
	Hepatocytes	
	Chondrocytes	
	Neutrofils	
	Epithelial cells	
IL-9	T cells	Mast cell enhancing activity
		Stimulates hematopoiesis
		Enhances T-cell survival *in vitro*
IL-10	T cells	Inhibits antigen presentation by macrophages
	B cells	Inhibits macrophage proinflammatory cytokine production
	Monocytes/macrophage	Induces B-cell activation, Ig synthesis
	Keratinocytes	Increases antibody responses
	Dendritic cells	
IL-11	Fibroblasts	Synergistic activity with IL-3 and IL-4 in megakaryocyte development
	Stroma fibroblasts	Stimulates acute-phase protein production
IL-12	B cells	Stimulates T-cell differentiation to T_H1 cells
	T cells	Stimulates NK-cell growth and activation
	Macrophages	
IL-13	T cells	B-cell growth and differentiation
		Inhibits monocyte inflammatory cytokine production
		IgG4 and IgE switch
IL-14	T cells	Induc s growth of activated B cells
		Inhibits Ig production by activated B cells

TABLE 9.1. *Continued*

Cytokine	Producer cells	Main actions
IL-15	T cells	Induces B-cell growth and differentiation
	Bone marrow stroma	Induces T-cell proliferation
IL-16	T cells	$CD4^+$ T growth factor
		Chemotactic factor for T $CD4^+$ cells
IL-17	T cells	Induces IL-6 and IL-8 production by fibroblasts
		Increases adhesion molecule expression by fibroblasts
IL-18	Phagocytic cells	Augments IFN-γ production by T cells, NK cytotoxicity, and T-cell proliferation
IFN-α/β	T cells	Antiviral activity
	B cells	Stimulates macrophage functions
	Monocytes/macrophages	Regulates MHC class I and II expression
	Fibroblasts	
IFN-γ	T cells	Macrophage activation
	NK cells	Increases MHC expression
G-CSF	T cells	Stimulates neutrophil activation and differentiation
	Macrophages	Stimulates differentiation of granulocyte-macrophage colony
	Neutrophils	
	Endothelial cells	
	Fibroblasts	
GM-CSF	Macrophages	Stimulates stem cell development
	T cells	Stimulates differentiation of myelomonocytic lineage
	Endothelial cells	
	Mast cells	
	Neutrophils	
	Eosinophils	
	Fibroblasts	
TGF-β	Chondrocytes	Stimulates extracellular matrix proteins
	Osteoblasts	Tissue repair
	Osteoclasts	Activates osteoblasts and inhibits osteoclasts
	Platelets	Inhibits NK functions
	Fibroblasts	Inhibits T- and B-cell proliferation
	Macrophages	Synergistic action with IL-4 in IgA secretion
	NK cells	
	Hepatocytes	
TNF-α	Neutrophils	Regulates the gene expression of growth factors, cytokines, transcription factors,
	Activated lymphocytes	receptors, and acute-phase proteins
	NK cells	Helps resistance to infections and neoplasm growth
	LAK cells	Local inflammation
	Astrocytes	Endothelial activation
	Endothelial cells	
	Smooth muscle cells	
TNF-β	Lymphocytes	Functions similar to TNF-α

CM-CSF, granulocyte–macrophage colony–stimulating factor; G-CSF, granulocyte colony–stimulating factor; IL, interleukin; IFN, interferon; LAK, lymphokine-activated killer; MHC, major histocompatibility complex; NK, natural killer; TGF, transforming growth factor; T_H, help T cell; TNF, tumor necrosis factor.

Cytokine receptors share a number of characteristics. Many of them are multisubunit structures that bind ligands and act as signal transducers because of their intrinsic tyrosine kinase activity. Several receptors often share common signal-transducing receptor components in the same family, which in part explains the functional redundancy of cytokines. This redundancy and the ubiquitous cellular distribution of certain cytokine receptors have hampered attempts to define critical responsive cell populations and the physiologically important cell-specific functions of cytokines *in vivo* (21).

T_H1 AND T_H2 CYTOKINES

Since 1986, when Mosmann et al. reported the existence of two different $CD4^+$ T cells based on the cytokines they produce, the T_H1/T_H2 model has evolved to encompass several newly discovered cytokines and major new functions (22–24). T_H1 cytokines and T_H2 cytokines refer to the patterns of cytokines secreted by two subpopulations of murine $CD4^+$ T cells that determine the outcome of an antigenic response toward humoral or cell-mediated immunity.

Cells other than T cells expressing CD4 are capable of producing T_H1 and T_H2 cytokines. These cells include $CD8^+$ T cells, monocytes, natural killer (NK) cells, B cells, eosinophils, mast cells, basophils, dendritic cells and other cells. T_H1 cytokines include IL-2, IFN-γ, IL-12, IL-18, and TNF-β. T_H2 cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13 (25,26).

The most potent cytokine inducer of T_H2 cells is IL-4. Several types of immune cells can produce IL-4 in the initial

immune response, including NK1.1$^+$CD4$^+$ T cells, γδ T cells, CD8$^+$ T cells, and mast cells (27,28).

IL-10 inhibits the induction of T_H1 immunity and enables the elaboration of a T_H2 response. Because IL-10 can be produced by certain APCs (15,29), it has been hypothesized that, under the influence of transforming growth factor-β (TGF-β), the preferential production of IL-10 by APCs leads to the development of T_H2 immunity in autoimmune diseases. It has been hypothesized that the autoregulatory effects of IL-10 on APC function might be a critical factor in the ability of an APC to present antigen in a suppressive manner (29).

In direct contrast to IL-4 or IL-10, IL-12 strongly induces the development of T_H1 cells (30). Activation of monocytes/macrophages usually results in elaboration of the proinflammatory cytokine IL-12 (30). One effect of IL-12 is direct stimulation of T_H1 differentiation. Another effect is the stimulation of IFN-γ production by T and NK cells. IFN-γ is a potent inducer of T_H1 immunity by the additional stimulation of IL-12 secretion and the inhibition of IL-4 (24,25,31).

T_H1-cell development depends on IL-12 produced by APCs. The interaction of the CD40 ligand on T cells with CD40 on dendritic cells results in very high production of IL-12 (32,33), favoring the development of a T_H1 response. Because of the potent proinflammatory and immunoregulatory functions of IL-12, the immune system has developed feedback mechanisms for antagonizing its action. IL-10 negatively regulates IL-12 p40 transcription. In B-cell lines, the nuclear factor NF-κB appears to play a crucial role in the regulation of IL-12 p40 production. Molecular analysis of the promoter region of the human gene for p40 has also identified a member of the ETS family of transcription factors as a major regulatory factor (32).

Another exogenous factor also influencing the development of undifferentiated CD4$^+$ T cells toward the T_H1 or T_H2 phenotype is TGF-β (34). Murine T_H2 cells, but not T_H1 cells, also express P600, the human counterpart of which has been identified as IL-13 (35). A novel cytokine inducing the synthesis of IFN-γ in T_H1 cells has been identified as IL-18 (36).

T_H1 and T_H2 cells not only produce a different set of cytokines but also appear to express different activation markers preferentially. CD30, a member of the TNF receptor superfamily, is mainly expressed by T_H2-like and cytotoxic T cells, whereas the product of lymphocyte activation gene-3 (LAG-3), a member of the immunoglobulin superfamily, preferentially associates with T_H1-type cells (37). It has been identified a stable cell-surface marker (STL2) expressed on T_H2 but not T_H1 (38).

The different patterns of cytokine secretion correspond with different functions as immune effectors. T_H1 cells promote cell-mediated effector responses. T_H2 cells are mainly helper cells that influence B-cell development and augment humoral responses such as the secretion of antibodies, predominantly of Ig E, by B cells. Both types of helper T cells influence each other by the cytokines they secrete; IFN-γ, for example, can downregulate T_H2 clones, and T_H2 cytokines, such as IL-10, can suppress T_H1 functions. IFN-γ can inhibit the proliferation of murine T_H2 cells but not that of T_H1 clones. It appears that these functional subsets are mutually antagonistic such that the decision about which subset predominates within an infection may also determine its outcome.

T_H1-type and T_H2-type responses in humans may play an important role in certain diseases. T_H1-type responses are involved in the pathogenesis of organ-specific autoimmune disorders and acute allograft rejection and in some chronic inflammatory disorders of the gastrointestinal tract, such as gastric antritis and Crohn's disease. In contrast, allergic reactions involving IgE and mast cells result from the development and activation of allergen-specific T_H2 cells. Different types of helper T-cell populations resembling those observed in mice are found also in humans. However, the differences in cytokine expression seem to be quantitative rather than qualitative.

CYTOKINES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Immune Dysregulation

Systemic lupus erythematosus (SLE) is the prototype of human autoimmune disease, characterized by multisystem involvement and autoantibodies to nuclear, cytoplasmic, and cell-surface autoantigens. The mechanism responsible for the breakdown of self-tolerance is unknown. This disease has a multifactorial pathogenesis, with genetic and environmental precipitating factors. The immune dysregulation that characterizes the disease is complex but has two main characteristics. One is B-lymphocyte hyperactivity and immunoglobulin repertoire changes, causing increased spontaneous production of immunoglobulins and autoantibody production (39). The mechanism by which B lymphocytes remain inappropriately activated for years during SLE or other autoimmune diseases are still poorly understood and may involve several agents acting in concert: genetic background, environmental factors such as drugs, hormonal influences, viral infections, and abnormal production of cytokines (40–42). The other is an impaired cell-mediated immunity. This includes decreased T-cell proliferative responses in autologous mixed lymphocyte reactions or after stimulation with mitogens, defective costimulatory signals, and several defects in the production of cytokines. The impaired cell-mediated immunity results from T-lymphocyte and APC dysfunctions (43–45).

Cytokine Regulation

Since 1982, when we reported a defect in the production of and response to IL-2 by mononuclear cells from SLE patients, the production of cytokines in this disease has become the object of numerous studies (46–48). Cytokines have been suggested to play an important role in the immune dysregulation observed in SLE patients and murine lupus-prone strains. Some of the T-cell abnormalities found in SLE patients can be attributed to decreased activity of IL-2.

Whether caused by an intrinsic T-cell alteration or not, a defect in IL-2 regulation occurs in patients with SLE and contributes to the complex disturbances of the immune system of SLE patients.

Although the origin of this abnormality is unknown, several mechanisms have been proposed to explain it: a primary defect of CD4 cells; a suppressive effect of the activity of IL-2 by CD8 cells by means of specific antibodies against IL-2 or by other cytokines; exhaustion of T cells because of their activation *in vivo*; and deficiency of other cytokines that participate in T cells activation. It was later shown that this was not an intrinsic defect, because it recovered after the cells were rested (49). It was also shown that SLE monocytes had defective production of (47,49) and response to IL-1 (49).

Multiple cytokine-mediated alterations have been demonstrated in SLE patients. Abnormal (increased or decreased) production of IL-1, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, TGF-β, and IFN-γ have been reported (50). Moreover, some of these cytokines (IL-1, TNF-α, IL-6, and IFN-γ) have been found in renal tissue of these patients, suggesting a local pathogenic effect (51).

Although peripheral blood mononuclear cells from SLE patients produce decreased amounts of IL-6 on stimulation, B lymphocytes from SLE patients spontaneously produce IL-6 and constitutively express IL-6 receptors (52). *In vitro* inhibition of this autocrine loop by anti-IL-6 receptor antibodies decreases the spontaneous production of autoantibodies. Anti-TNF-α antibodies also decrease production of immunoglobulins by cultured peripheral blood mononuclear cells of SLE patients, but circulating TNF-α is not detected in such patients. A correlation has been demonstrated for the serum levels of TNF-α receptors, IL-1 receptor antagonist (IL-1ra), and clinical disease activity. Overexpression of TNF-α mRNA in bone marrow cells also has been observed in these patients (53).

The elevated levels of TNF-α and IL-10 observed in SLE patients reflects the hyperactivation of cells that are responsible for the overproduction of autoantibodies. Once induced, these cytokines act as proinflammatory effectors through their biologic properties, including stimulation of a cascade of other soluble factors that amplify the initial stimulus and lead to the progression of the disease. Abnormal IFN-α production has been found in SLE patients (54). This cytokine has pleiotropic effects on the immune system, and its defective production can induce autoimmune manifestations. It has a role in upregulation of major histocompatibility complex (MHC) class II molecules and in this way contributes to the pathogenesis of autoimmunity.

The B-cell hyperactivity found in SLE and in other autoimmune diseases may be related to a generic increase in T_H2 cytokines, an increase caused by IL-4, IL-6, and IL-10. However, the IL-6 production in SLE may result from the hyperactivity of cells other than T cells, and increased IL-10 production results from monocytes and B cells rather than T lymphocytes (55). The autocrine and paracrine effects of IL-10 may be crucial to the B-cell hyperactivity and particularly to autoantibody production. IL-10 is a potent stimulator of B lymphocytes, and it stimulates the production of anti-DNA autoantibodies by peripheral blood mononuclear cells from SLE patients (56). It is also a potent inhibitor of APC and T-lymphocyte functions. Increased production of IL-10 could explain the two main characteristics of the immune dysregulation of SLE. Consistent with this concept, several reports demonstrated increased production of IL-10 in SLE patients (57,58).

Immune dysregulation partially mimicking that of SLE has been described in healthy relatives of SLE patients. Some of these relatives display autoreactive B-lymphocyte hyperactivity, although the autoantibodies produced are of low affinity and are not pathogenic. A larger proportion of the relatives display impaired cell-mediated immunity, decreased IL-2 production, and polyclonal B lymphocyte hyperactivity (59). Relatives of SLE patients have a dysregulation of IL-10 production similar to that of patients. This finding strongly suggests that IL-10 gene dysregulation may belong to the background predisposing to the disease rather than representing a simple marker of immune activation (60).

IL-12 is another cytokine that appears to play a role in polyclonal B-cell activation observed in SLE. Its presence in mononuclear cell cultures is able to downregulate the spontaneous immunoglobulin production; this inhibiting activity does not seem to be mediated through IFN-γ secretion (61).

The decreased production of lymphocyte-derived TGF-β in SLE cannot be normalized by the addition of IL-2 and TNF-α or by antagonism of IL-10. Abnormal production of each of these cytokines in SLE could be important in the perpetuation of B-cell hyperactivity (62).

CYTOKINES IN RHEUMATOID ARTHRITIS

Pathogenesis of Rheumatoid Arthritis

Although much work has been done, the pathogenesis of rheumatoid arthritis (RA) remains obscure. There are well-recognized susceptibility factors that involve hormonal factors that could account for the disease predominance in women and the genetic contribution to the disease that is clearly contained within the class II MHC locus, especially in the DR1 and DR4 disease-susceptible haplotypes. These haplotypes are found in more than 80% of RA patients of Caucasian origin and constitute one of the strongest pieces of evidence to support the theory that T lymphocytes are important at some point in the pathogenesis of RA by shaping the T-cell receptor (TCR) repertoire or in the presentation of an inducing microbial or autoantigenic peptide. Although many infectious agents have been implicated in the cause of RA over the years, including viruses, mycoplasmal organisms, and mycobacteria, none has been proved to be causative.

RA is considered to be a heterogeneous and systemic disease; it extends throughout the synovial joint and in some cases well beyond the joint. One characteristic finding in RA synovitis is the increase in cellularity, which is most evident

in the synovial membrane that becomes infiltrated by cells thought to be recruited from the blood. Characteristically, the lining layer (intima) increases from one to two cells thick to a layer six to eight cells thick composed mostly of activated type A synoviocytes (i.e., macrophage type) along with type B synoviocytes (i.e., fibroblast type). In rheumatoid synovitis, there is also formation of lymphoid aggregates resembling germinal centers in the deeper portions of the synovium around blood vessels containing activated endothelial cells that form high endothelial venules, the site where most activated T cells extravasate (62,63). The most abundant cells in the synovial membrane during the early stages of the disease are macrophages and T lymphocytes, but plasma cells, dendritic cells, and activated fibroblasts are also found. This histologic picture changes according to the chronicity of the disease; in later stages, the type B synoviocytes and macrophages predominate, with fewer number of T cells (64). Many of these cells are activated and express abundantly class II human leukocyte antigen (HLA) and adhesion molecules of relevance in antigen presentation (65–67).

The histologic hallmark of RA, the pannus, is the major site of irreversible tissue damage and originates at the junction of the synovium lining the joint capsule with the cartilage and bone. This tissue is rich in transformed type B synoviocytes and macrophages. The cells of the pannus invade over the underlying cartilage and into the subchondral bone, causing the erosion of these tissues that is characteristic of the disease (68). Cartilage destruction results from the activity of matrix metalloproteinases (MMPs), which are proteolytic enzymes produced and secreted by macrophage-type synoviocytes and type B synoviocytes in response to proinflammatory cytokines such as IL-1 and TNF-α. Collagenase (MMP-1) and stromelysin-1 (MMP-3), whose production is increased, have been found to be important in the destructive process in RA (69). The activity of MMP is regulated to some extent by tissue inhibitors of metalloproteinases (TIMPs); these bind irreversibly the enzyme to form a 1 : 1 complex with the MMP. These TIMPs are produced predominantly by type B synoviocytes and to a lesser extent by the macrophage-type synoviocytes. Two immunoregulatory cytokines, TGF-β and IL-10, that are produced in RA synovium inhibited the production of proinflammatory cytokines that induce MMPs and induced the production of their natural inhibitors, TIMPs (70,71).

Cytokine Expression in Rheumatoid Arthritis

Much of the initial data reporting the levels of cytokines were obtained from studies performed in the synovial fluid of RA patients. Many studies investigated a limited number of cytokines from each joint (72,73), and as such, the production of IL-1 was first documented in this compartment (74). The relevance of cytokines initially found in the synovial fluid to the pathogenesis of the disease is unclear. The synovial fluid is composed of a complex mixture of molecules, including a large concentration of hyaluronan, other proteoglycans, serum proteins, and degradative enzymes, many of which inhibit or degrade cytokine function. The information gained from these studies therefore is unlikely to be of relevance to the pathogenesis of RA.

Cytokine expression in synovium probably is of greater relevance to the origin of RA, because most investigators agree that this is the principal site of immune and inflammatory activity. Cytokines interact, and it has been suggested that they should be considered as a network (75). The importance of simultaneously detecting multiple cytokines is illustrated by T-lymphocyte cytokines, for which patterns of cytokine production have profound implications to the outcome of immune responses. Although *in vivo* evidence shows that few T cells secrete the very restricted cytokine patterns originally described as T_H1 and T_H2 subsets (76,77), it is clear that cell-mediated immune responses are dominated by T_H1 cytokines such as IFN-γ and humoral responses by T_H2 cytokines such as IL-4 (78). This subject is covered in more detail elsewhere in this chapter.

The initial data on cytokine expression in the synovial membrane in RA were generated by Northern or Southern hybridization, which provided abundant information on the different cytokines expressed in RA synovium. However, these techniques have been replaced by more sensitive methods such as reverse transcriptase–polymerase chain reaction (RT-PCR). The RT-PCR provides a useful method for detecting the expression of a large number of cytokine mRNA species from sites of human disease that provide limited sample size. This technique is useful for detecting mRNA expressed at low levels, such as T-lymphocyte mRNA. Most of the RA tissue used in these studies has come from operative joint replacement, a procedure that usually is done during the late stages of disease. The tissue samples therefore can be expected to have a different cellular profile from that of early-stage disease.

Proinflammatory Cytokines

IL-1 and TNF-α protein were readily detected in synovial fluid (79–81). In the synovial tissue at the mRNA levels, these cytokines can be detected by blotting and by *in situ* hybridization (82). Immunostaining with monoclonal antibodies specific for these cytokines demonstrated expression predominantly in macrophage-type synoviocytes (83). Later, they were also detected in short-term culture of synovial cells, and IL-1 and TNF-α were detected in a bioassay of synovial membrane cultures; they were present in quantities able to signal a biologic response effectively (84). Laboratory and clinical evidence suggest that TNF-α has a particularly relevant role in the pathogenesis of RA (85,86). TNF-α induces the release of MMP from neutrophils, synovial fibroblasts, and chondrocytes (87–89); induces the expression of endothelial adhesion molecules involved in the migration of leukocytes to extravascular sites of inflammation (90); and stimulates the release of other proinflammatory cytokines, specifically IL-1 secretion (84,91).

TNF-α concentrations are increased in the synovial fluid of patients with active RA (79,83), and increased plasma levels of this cytokine are associated with joint pain (92). Later, as other proinflammatory cytokines and growth factor complementary DNA (cDNA) were cloned, it became important to study the presence and relevance of TNF-α in RA, and the mRNA and protein were detected initially in RA synovial fluid. Among these cytokines were IL-6 (93–95), interferon-α (IFN-α) (81), granulocyte-macrophage colony-stimulating (GM-CSF) (96), macrophage colony-stimulating factor (M-CSF) (97), and leukemia inhibitory factor (LIF) (98–100). Most studies used osteoarthritis tissue or fluid as controls, and usually the same array of cytokines were produced, although at a lower level.

IL-6 derived from synovial fibroblasts during active disease has been established as the cytokine that induces the hepatic synthesis of acute-phase proteins in RA (101). Interleukin-12, a monocyte derived proinflammatory cytokine is a potentially relevant cytokine in RA, because it has a role in skewing the immune response toward T_H1 (102). It is likely that IL-12 could be involved early in the RA process, when $CD4^+$ T_H1 lymphocytes predominate in the inflammatory infiltrate. In later stages of the disease, IL-12 appeared to be important in maintaining the T_H1 preponderance in the immune response and in controlling cytokine production; this was confirmed in studies of RA patients (103,104).

The cells that form most of the hyperplastic pannus are the type B or fibroblast-type synoviocytes, and the cytokines identified as major contributors in this hyperplasia include platelet-derived growth factor (PDGF) (105,106), fibroblast growth factor (FGF) (105,107), and TGF-β (106,108–110). Which of these predominantly drives the hyperplastic response is debatable, but the data point to a additive effects of PDGF and FGF. Later data concerning the role of the newly cloned IL-15, which has an IL-2 activity, demonstrate its participation in RA. Studies showed that IL-15–activated T cells stimulated the secretion of TNF-α by macrophages through a cell-contact–dependent mechanism, and this effect was seen in peripheral blood T cells and in synovial T cells from RA patients (111). These findings are interesting because IL-2 has the opposite effects in this study, despite sharing the γ chain of their receptors.

In another study, IL-15 was found to be a chemoattractant for T cells in RA, and its expression was demonstrated by immunostaining in the lining layer of the synovial membrane. These investigators (112) also found that synovial fluid T lymphocytes proliferate in response to IL-15, demonstrating that continued responsiveness to IL-15 is a feature of T cells after entry into the synovial compartment. The investigators concluded that IL-15 can recruit and activate T lymphocytes in the synovial membrane, thereby contributing to RA pathogenesis (112). These findings need to be confirmed before considering IL-15 as the predominant cytokine in directing the immune response in RA.

Chemokines

The prominent features of the rheumatoid synovial microenvironment, such as the selective accumulation of memory T cells bearing the activation markers $VLA4^+$ and $CD45RO^+$ and activated macrophages in the membrane and of polymorphonuclear cells in the fluid, suggest an important role for leukocyte chemoattractant molecules such as chemokines. The superfamily of chemokines consists of an array of cytokines unparalleled in biology: the current roster approaches 50 related proteins. These proteins range in size from 68 to 120 amino acids (in the natural form) and can be conveniently divided into at least three structural branches: C, CC, and CCC, according to variations in a shared cysteine motif (113). The largest branch, that of the CC or β chemokines, has nearly 20 members in humans. The smallest branch, the C class, has but one. The CXC is a chemokine branch can be further subdivided by structure and function. Further dissection of this still growing family of cytokines is beyond the scope of this chapter, but the topic is reviewed elsewhere (114).

Chemokines are released by the cells present in abundant numbers in RA, including endothelial cells, synovial fibroblasts, macrophages, and lymphocytes. Members of all three chemokine superfamilies have been implicated in the pathogenesis of RA. The first chemokine to be described in RA was IL-8 (115), and it initially was correlated with the increased angiogenesis common in early stages of the disease. Using immunohistochemical analysis, several groups reported the expression of epithelial cell–derived neutrophil-activating peptide-78 (ENA-78) (116), macrophage inflammatory protein-1α (MIP-1α) (117), monocyte chemotactic protein-1 (MCP-1) (118), and regulated on activation, normally T cell expressed and secreted (RANTES) (119), predominantly associated with synovial tissue macrophages and, to a lesser extent, with the activated endothelium (i.e., high endothelial venules) and type B synoviocytes.

Chemokine Receptors

Chemokine receptors are members of the large family of serpentine receptors with seven transmembrane domains that couple to *Bordetella pertussis* toxin-sensitive heterotrimeric G proteins for signal transduction (120,121). The two subfamilies of receptors, CXCR and CCR, interact with CXC or CC chemokines, and cross-selectivity has not been observed. Because the predominant leukocyte infiltration in RA is composed of T cells and macrophages, β chemokines are likely to be important in the accumulation of these cells in the RA synovium. Most T cells infiltrating the synovial membrane in RA are activated or memory T lymphocytes and particularly important for the migration of activated or memory T cells are the CXCR3 ligands IP-10 (IFN-γ–inducible 10-kd protein) and MIG (monocyte/macrophage-activating, IFN-γ–inducible protein) and the CCR5 ligands RANTES, MIP-

1α, and MIP-1β. It was demonstrated by inmunostaining of T cells in RA synovial fluid that virtually all such T cells expressed CXCR3 and about 80% expressed CCR5, representing a high enrichment over levels of $CXCR3^+$ and $CCR5^+$ T cells in peripheral blood, which were 35% and 15%, respectively (122). The investigators concluded that these results demonstrate that the chemokine receptors CXCR3 and CCR5 are markers for T cells associated with certain inflammatory reactions, particularly T_H1-type reactions, as is seen in RA (52; B. Moser, personal communication, 1999).

Identifying the nature of this lymphocyte subset migration is important for understanding the cellular and molecular mechanisms of inflammation and for designing strategies for selective immunosuppression. These are encouraging results for identification of a particular subset of potentially pathogenic T cell in RA, although we still need to determine whether the actions of IP-10 or MIG, resulting in the recruitment of $CXCR3^+$ lymphocytes, are the reason for the distinctive phenotype of migrating cells or whether another chemokine such as a CCR5 ligand is responsible. An alternative explanation for the high expression of CXCR3 or CCR5 on inflammatory cells is upregulation by inflammatory cytokines after extravasation, an environment existing in RA.

Antiinflammatory Cytokines

Among this group of cytokines, the ones with proved relevance to RA are TGF-β, IL-4, IL-10, and IL-13. Several groups have reported TGF-β to be abundant in its precursor, inactive form and in the active form in rheumatoid synovium (123–125). However, there is controversy about the role of TGF-β in RA, because studies using animal models of joint inflammation have shown a proinflammatory effect instead of an antiinflammatory one (126). In another study, the administration of anti-TGF-β into the joints of rats with arthritis decreased the inflammation (127). This cytokine is likely to be important in the reparative and fibrotic process in the joints because it inhibits the production of MMPs such as collagenase (123), induces TIMP (128), and stimulates the production of type I and type XI collagen. Locally secreted TGF-β may promote reparative processes in arthritic synovial connective tissue and tissue repair by inhibiting cartilage and bone destruction. The disruption in the balance between these activities would result in different outcomes of the initial stimulus for TGF-β secretion. In chronic lesions, the overproduction of TGF-β could participate in the ongoing damage by recruiting inflammatory macrophages and activating synovial fibroblasts with the potential for tissue destruction.

Initial studies of the expression of IL-4 demonstrated that this T-cell–derived antiinflammatory cytokine is absent in the RA synovium (129). This indicated that the T_H2 pattern of cytokines is not abundant in RA and that the T_H1 type predominates in this disease (130). However, with the advent of more sensitive techniques such as PCR, some investigators were able to detect IL-4 mRNA (131–133). The net effect of this low level of IL-4 production in the RA joint is unknown, although some researchers have proposed this virtual lack of IL-4 in RA as a potential therapeutic target for these patients (134).

IL-10 is regarded as a T_H0 cytokine, because it has profound antiinflammatory and immunoregulatory effects, and it is secreted in a subset of human T cells that differentiate under the influence of IL-12 into an IFN-γ/IL-10 subset (135). It has been extensively documented in RA peripheral blood (55) and synovial joints by RT-PCR of biopsy specimens and identified in synovial cell culture of RA patients (136,137). Among its antiinflammatory effects relevant to RA are inhibition of IL-1β secretion (although IL-4 is more potent in this effect), induction of soluble TNF-α receptors (TNFRs) production, and downregulation of the membrane-bound TNF-α receptor (138), producing a net effect of less biologic activity for TNF-α. Among the proinflammatory activities is stimulation of B-cell activity, which may be important in driving the production of rheumatoid factor (139), and possible prevention of apoptosis of B and T lymphocytes (72,140). Because IL-10 is abundant in the RA joint, it may have a role in sustaining the survival of T cells there.

IL-13, a product of activated T cells, has multiple biologic actions, primarily on B cells and monocytes. It inhibits the production of proinflammatory cytokines, chemokines, and hematopoietic growth factors by activated human monocytes. In studies of RA patients, it has been found to be consistently produced in synovial fluid lymphocytes, and IL-13 levels were significantly higher than those of IL-4 (140). The investigators also demonstrated *in vitro* the inhibitory effect of IL-13 in the secretion of IL-1β and TNF-α by synovial fluid macrophages from RA subjects. These findings suggest that IL-13 may have a therapeutic potential in the treatment of patients with RA, although they should be confirmed first.

Cytokine Regulation in Rheumatoid Arthritis

Since the start of studies of cytokine expression in RA, a different pattern of cytokine regulation emerged from that found for *in vitro* activated cells. An important distinction was the consistent pattern of cytokine production, with virtually all samples analyzed producing essentially the same pattern; the level of IL-1α was relatively high, in contrast to *in vitro* stimulated macrophages, in which IL-1β predominates over IL-1α) (142). The presence of cytokines in all rheumatoid synovial membrane samples suggested that, unlike what is reported in normal cells stimulated *in vitro*, in which cytokine expression is transient, cytokine expression in rheumatoid synovium probably was prolonged or continuous. The signal that drives this cytokine pattern in RA was later found to be TNF-α (84).

TNF-α was shown to be the major regulator of IL-1 in RA synovium and of other relevant proinflammatory cytokines

such as GM-CSF, which is responsible for maintaining the increased MHC class expression on RA synoviocytes (96). TNF-α emerged as the key cytokine regulator of the pro-inflammatory cytokine cascade. What regulates TNF-α production in RA joints is remains unsolved, although some data from treatment studies suggest that the process may be T-cell mediated, because depletion of T cells in the joint leads to decreased TNF-α production. However, the specific T-cell–derived signals to the monocytic cells, the principal source of TNF-α, remains unknown. The important role of TNF-α in the pathogenesis of RA was subsequently confirmed in therapeutic clinical trials in which the biologic effects of this cytokine were affected. The first strategy was to use neutralizing monoclonal antibodies, anti-TNF-α. With the intent of diminishing the risk of immunogenicity, the trials used a biologic treatment consisting of a chimeric monoclonal antibody (75% human immunoglobulin). This monoclonal antibody (cA2) was effective in improving all indices of disease activity used to monitor the study subjects. The indices included relevant validated outcome measures such as the number of swollen and tender joints and laboratory evidence of inflammatory activity such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (143) levels. The cA2 effects lasted a median of 12 weeks in this study. The magnitude of the clinical response with this agent was convincingly reproduced in a randomized, double-blind, placebo-controlled trial (144). During follow-up of these patients, important adverse effects were found; infectious episodes were increased compared with the placebo-treated group, although the investigators reported the infections were not life threatening. An unexpected development of IgM-class anti-dsDNA antibodies was documented in 6% the cA2 treatment group without clinical SLE, and in all cases the antibody levels resolved over several months. The origin and potential pathogenicity of these antibodies remain unsolved.

To exert a more selective blockade of the TNF-α cascade in another study, RA patients were treated with a recombinant TNFR p75-Fc fusion molecule (145). This approach was based on the knowledge that there are two distinct cell-surface TNF-α receptors, designated p55 and p75 (146,147). Soluble, truncated versions of the membrane TNFRs, consisting of only the extracellular, ligand-binding domain, are present in body fluids and are thought to be involved in regulating TNF activity (148,149). These soluble TNFRs have been detected in synovial tissue and at the junction between cartilage and pannus (150,151), and their levels are increased in the serum and synovial fluid of RA patients (152–155). The results of the randomized, double-blind trial with TNFR p75-Fc fusion protein (145) in RA patients demonstrated its efficacy. Treatment with TNFR-Fc for 3 months reduced disease activity as assessed by a number of clinical end points, biochemical markers of disease, and quality of life reports in this trial. The investigators postulated that the mechanism of action of this biologic agent probably involves its ability to inhibit competitively TNF-α binding to cell-surface TNFR. An important advantage was that patients so treated did not develop antibodies that neutralized the therapeutic agent, possibly because the chimeric protein contained only human amino acid sequences. The researchers observed only minor adverse effects, such as injection-site reactions and mild upper respiratory symptoms such as cough, rhinitis, and pharyngitis. Overall, the safety-efficacy profile of TNFR-Fc seems promising. Further long-term studies are needed to establish its role in the contemporary treatment strategies for RA.

IL-1 activity is modulated by IL-1 receptor antagonist (IL-1ra), the only cytokine receptor antagonist known (156). IL-1ra has a high affinity for type I and II membrane IL-1 receptor, but because of the ability of IL-1 to activate cells at very low receptor occupancy rates, a high molar excess (about 100 : 1) of IL-1ra is required to antagonize the biologic activity of IL-1. Expression of IL-1ra is upregulated at mRNA and protein levels in RA (157) and is localized to $CD68^+$ macrophages within the synovium. The ratio of IL-1ra to IL-1 in RA is 1.2 : 3.6 (158), well below the 100-fold excess required to neutralize IL-1 bioactivity, which produces the net effect of increased bioactive IL-1 in RA patients.

CYTOKINES IN INSULIN-DEPENDENT DIABETES MELLITUS

Insulin-dependent diabetes mellitus (IDDM) is an organ-specific autoimmune disorder in humans and results from the inflammatory destruction of insulin-secreting pancreatic β cells. The clinical onset of diabetes is preceded by periinsulitis and insulitis. Insulitis is characterized by infiltration of the pancreatic islets of Langerhans by T cells, B cells, and macrophages (159,160). Genetic and environmental factors (e.g., viral infections) play a role in the development of this highly prevalent autoimmune disease. The various stages leading to the destruction of insulin-secreting cells probably take place over months or years in humans, because immunologic markers of antiislet autoimmunity (i.e., autoantibodies directed against islet antigens like antiglutamate decarboxylase [GAD] or antiinsulin) can be detected well before clinical onset of the disease. This long preclinical phase suggests the possibility of effective development of prophylactic treatment to delay or halt progression toward insulin deficiency. However, to provide intervention at the adequate moment through appropriate therapeutic measures requires an understanding of the immunologic mechanisms controlling the various stages of the autoimmune process and their chronology. We emphasize the data relevant to human IDDM, but animal models are mentioned when deemed necessary.

Evidence suggesting a role of functionally polarized T cells, differing by their cytokine secretion patterns, in regulating immune responses directed against a variety of antigens has provided the framework for a new hypothesis concerning the development of autoimmunity in IDDM. The possibility that an imbalance between the helper T lymphocytes (T_H1 and T_H2) favors the activation of immune effectors against insulin-secreting cells remains controversial.

Most studies using experimental models favor the role of T_H1 lymphocytes in the development of autoimmunity to pancreatic β cells (161–163). Whether such a T-cell imbalance is directly responsible for triggering the autoimmune process is uncertain, but an attractive approach to interrupting the harmful autoimmune process and providing protection against the disease is to artificially favor the activation of T_H2 over T_H1 regulatory T cells.

T_H1 and T_H2 Lymphocyte Subtypes

Effector functions in the immune system are carried out by lymphocytes, which produce antibodies, and T lymphocytes, which secrete a variety of cytokines and focus cytolytic activity on target cells in response to recognition of processed antigens. The various helper T-cell subsets were initially identified in cloned murine T cells (161) and subsequently characterized in humans (162). The current concept of helper T-cell subsets in humans is that T_H1 cells promote inflammatory cellular immune responses and are biased toward secretion of IFN-γ, IL-2, and possibly TNF-β. T_H2 cells are biased toward secretion of IL-4, IL-5, IL-6, IL-10, and IL-13; induce humoral immunity; and inhibit T_H1 responses.

Studies of human autoimmune diseases such as Crohn's disease, multiple sclerosis, Graves' ophthalmopathy, and Hashimoto's thyroiditis have demonstrated a T_H1 cytokine profile. Conversely, skin T cells in systemic sclerosis are T_H2 (164). The role of T_H1 and T_H2 cells in T-cell regulation raises a number of issues in autoimmunity, especially in IDDM, a model of spontaneous autoimmune disease in animals (nonobese diabetic [NOD] mouse and biobreeding [BB] rat) and humans. Is there an initial imbalance during the early phase of autoimmunity or a progressive switch from a T_H2 to a T_H1 response that may offer a clue to disease development? Kinetics of the autoimmune process in human IDDM and the chronology of events leading to diabetes are considerably less well known in view of the lack of histologic access to the pancreas. Autoantibodies directed against pancreatic antigens (e.g., antiglutamate decarboxylase, antiinsulin), although highly predictive of diabetes outcome, are detected months to years before clinical onset of diabetes (165,166), but it has not been possible to correlate the presence of autoantibodies with the process that leads to the selective destruction of pancreatic β cells. Some subjects who carry antibodies never develop diabetes, and others lose autoantibodies with time and correct anomalies of carbohydrate metabolism (167).

Characterization of Helper T Lymphocytes Involved in Insulin-Dependent Diabetes Mellitus

Several lines of evidence suggest that the progression of the autoimmune process in the NOD mouse and the BB rat follows a T_H1 response profile. Most data indicate IFN-γ predominates over IL-4 gene expression and secretion within the lymphocytic infiltrate invading the pancreatic islets *in situ* or islets grafted under the kidney capsule in NOD mice (168–170), although not all investigators concur with these findings (171). A predominant T_H1 response in the IDDM process is further supported by the prevention of cyclophosphamide-induced diabetes in the NOD mouse by the injection of anti-IFN-γ antibodies (172) The transgenic expression of IFN-γ on β cells under the control of the rat insulin promoter has been shown to trigger the development of insulitis and diabetes in cases of conventional murine genetic backgrounds and the activation of islet-specific cytotoxic T cells (173,174). In a transgenic model in which a viral protein was expressed by β cells and diabetes was induced by systemic infection with the corresponding virus, the presence of IFN-γ was essential to disease development (175). However, inactivation of the IFN-γ gene in the NOD mouse delayed but did not prevent the onset of diabetes (176), indicating that compensating mechanisms can take over the T_H1 cytokine defect.

In humans, the systematic study of the pancreatic infiltrate is not readily accessible, but studies have shown the expression of IFN-γ in pancreatic tissue from patients with IDDM who died of ketoacidosis, and no T_H2 cytokines were detected (177). These difficulties in obtaining lymphocytes directly from human islet infiltrate and procuring human islet cells as a source of antigen have been major limitations in physiologic studies of islet-specific T lymphocytes. Studies performed after nonspecific activation of peripheral T cells by mitogens have uniformly reported a reduction in the ability of lymphocytes to produce IL-4 or a reduction in the IL-4/IFN-γ ratio compared with controls (178,179). Other studies performed after stimulation of peripheral T cells from recent-onset IDDM patients by human islet cells (140) or extracts of insulinoma membrane (180) have failed to detect IFN-γ secretion despite significant T-cell proliferation. However, these latter results were not replicated in a later study in which the levels of macrophage-derived cytokines such as IL-1, TNF-α, and IL-12 were measured in supernatants of stimulated peripheral blood lymphocytes in high-risk relatives of IDDM patients who had antiislet cell antibodies (i.e., anti-GAD and antiinsulin). The investigators found a correlation between the levels of antiislet antibodies and the levels of IL-12 produced by these subject T cells, supporting the view of T_H1 predominance in the early stages of the disease process (181).

In a study of a series of at-risk nonprogressors (defined by genetic susceptibility and the presence of antiislet antibodies) and IDDM patients (including five identical twin or triplet sets discordant for the disease), the diabetic siblings had a statistically significant lower frequency of $CD4^-CD8^-$ $Va24JaQ^+$ T cells compared with their nondiabetic siblings (182). These T-cell subsets are the initial source of IL-4. The investigators demonstrated that the few $CD4^-CD8^-$ $Va24JaQ^+$ T cells found in diabetic patients were biased toward a T_H1 cytokine pattern, secreting high levels of IFN-γ but negligible levels of IL-4, in sharp contrast to the high levels of IL-4 secreted by these T cells in nonprogressor siblings. This is the first study to clearly demonstrate that human IDDM is associated with an extreme T_H1 phenotype for

Va24JaQ$^+$ T cells and a decrease in their circulating frequency and indicates they may be functionally related to the resistance or progression of this autoimmune disease in humans.

Cytokines Secreted in Response to Islet Autoantigens

The autoantigens responsible for triggering the autoimmune reaction directed against insulin-secreting cells have not been definitely identified. Numerous antigens are involved in the course of the diabetic autoimmune process. Purified or recombinant GAD and insulin have been used to study the anti-β-cell T cell *in vitro*. In humans, islet cell antibodies are useful markers of autoimmunity to β cells. However, overwhelming evidence from animal models suggests that the autoimmune reaction is mediated by T lymphocytes and that the production of antibodies is a secondary phenomenon. Proliferation of mononuclear cells (including T lymphocytes) in the presence of GAD has been detected in the blood of recent-onset diabetic patients or subjects at risk for developing the disease (183). In at-risk individuals, an inverse relationship exists between the level of anti-GAD antibodies and detection of a proliferative T-cell response (184), implying the possible existence of a T_H1/T_H2 balance. However, cytokines produced by CD4$^+$ T lymphocytes on GAD recognition have not been studied. Conversely, GAD-specific cytotoxic T cells have been characterized in recent-onset diabetic patients as producing IFN-γ (185). Cytokine secretion accompanying T-lymphocyte responses to other pancreatic autoantigens has not been reported, possibly because of the low sensitivity of techniques used to measure cytokine production (e.g., ELISA) and the low precursor frequency of autoantigen-specific lymphocytes in the blood (186).

Immunotherapy and T_H1/T_H2 Balance in Insulin-Dependent Diabetes Mellitus

Studies performed in mouse models such as NOD support the idea of disease prevention or modulation by manipulating T_H2 responses through injecting IL-4 or interfering with T_H1 responses by administering anti-IFN-γ (172,187). Conflicting results have been obtained in mice by enhancing the expression of IL-10, which inhibits the production of T_H1 cytokines and proliferation of T_H1 lymphocytes induced by macrophages. However, transgenic expression of IL-10 in β cells accelerated development of insulitis and diabetes in the NOD model (188). In humans, immunotherapy with cytokines has not been attempted for IDDM. However, cytokines may prove nonspecific for modulating islet cell–specific T cells and favor deleterious immune reactions (e.g., viral reactivation, parasitic infiltration, allergic phenomena) (189). Although this has not been the case in the nonprogressors studied by Hafler who had extremely high levels of IL-4 in their blood and no evidence of immunosuppression or atopy, further follow-up is necessary to exclude this possibility.

Evidence supports the concept of IDDM being a T_H1-driven disease. It is still necessary to define the initial trigger of this biased response against the pancreatic β cells. Ongoing trials enlisting humans using orally administered insulin to prevent diabetes are based on the concept of antigen-driven bystander suppression. Regulatory cells elicited in the gut by orally administered antigens are thought to migrate to the pancreas, creating a tolerogenic environment and down-regulating the local inflammatory process. This protective mechanism seems to depend essentially on TGF-β. This cytokine produced by T_H1 or T_H2 cells has regulatory potential in T_H1- and T_H2-mediated autoimmune diseases (190).

CONCLUSIONS

Chronicity and destructive potential are characteristic features of the inflammatory response in the tissues of patients with rheumatic diseases. The past decade in research has been dominated by a shift from premolecular to molecular techniques. A major effort has been made to determine which cytokines and inflammatory mediators are produced at the site of disease. Tissue-residing and -infiltrating cells secrete proinflammatory cytokines *in situ*, which probably have a critical role in amplifying and maintaining inflammation (Fig. 9.1). The migration of inflammatory cells into the tissue is an important component of the disease; adhesion molecules facilitate tissue infiltration, and they affect cell activation and cell-cell and cell-matrix interactions.

The paradigm that RA is an antigen-driven and T-cell–mediated disease has prompted attempts to use T-cell–depleting reagents as therapeutic agents. Although T cells can be eliminated in peripheral blood, the overall therapeutic benefits have been minimal and accompanied by major side effects. The lack of therapeutic efficacy is combined with the persistence and selective proliferation of T cells in the joint, reemphasizing the role of tissue-infiltrating T cells in the disease. Studies of the composition of the T-cell infiltrate have demonstrated heterogeneity, indicating that the frequency of disease-relevant T cells is probably low. Pathologic T-cell function may be much more systemic than previously suspected. The relevance of modulation of the cytokine network in RA stimulated reevaluation of the mechanisms of action of established antirheumatic drugs, and methotrexate was found to increase the expression of IL-4 and IL-10 at the gene level in RA treated patients (159).

One of the remaining challenging questions being addressed is the possibility that better therapeutic results may be obtained in controlling RA by combination therapy. More than one cytokine could be targeted simultaneously, or a proinflammatory cytokine blockade could be combined with anti-T-cell therapy. A potentially useful approach could be the combination of the existing biologics with disease-modifying drugs of proven efficacy, such as methotrexate and Azulfidine or cyclosporine.

The many specific activities of individual cytokines have been the basis for current concepts of therapeutic interven-

tion, particularly the treatment of hematopoietic malfunctions and tumor therapy. Applications involve the support of chemotherapy and radiation therapy, bone marrow transplantation, and general immunostimulation. Although some cytokines are in clinical use, physicians must be aware that knowledge about them is limited and that the *in vivo* modulation of the activity of any one factor may not have the desired effect. Nevertheless, our new and growing understanding of the biologic mechanisms governing cytokine actions is an important contribution to medical knowledge. The biochemistry and molecular biology of cytokine actions explain some well-known and some obscure clinical aspects of diseases. Knowledge that cytokines create regulatory hierarchies and provide independent or interrelated regulatory mechanisms that can confer distinct and interactive developmental functions lays a solid, albeit rather complicated, foundation for current and future clinical experiences.

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